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PERK integrates oncogenic signaling and cell survival during cancer development

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Abstract

Unfolded protein responses (UPR), consisting of three major transducers PERK, IRE1 and ATF6, occur in the midst of a variety of intracellular and extracellular challenges that perturb protein folding in the endoplasmic reticulum (ER). ER stress occurs and is thought to be a contributing factor to a number of human diseases, including cancer, neurodegenerative disorders and various metabolic syndromes. In the context of neoplastic growth, oncogenic stress resulting from dysregulation of oncogenes such as c-Myc, $Braf^{V600E}$ and $HRAS^{G12V}$ trigger the UPR as an adaptive strategy for cancer cell survival. PERK is an ER resident type I protein kinase harboring both pro-apoptotic and pro-survival capabilities. PERK, as a coordinator through its downstream substrates, reprograms cancer gene expression to facilitate survival in response to oncogenes and microenvironmental challenges, such as hypoxia, angiogenesis, and metastasis. Herein, we discuss how PERK kinase engages in tumor initiation, transformation, adaption microenvironmental stress, chemoresistance and potential opportunities and potential opportunities for PERK targeted therapy.

Keywords

UPR; PERK; cancer; therapy

ER stress is intimately involved in a variety of human diseases, including diabetes, neurodegeneration, stroke, viral infection, and heart disease (Kim et al., 2008). Accumulating evidence suggests that chronic ER stress is readily apparent in multiple cancer types including melanoma, multiple myeloma, breast cancer, hepatocellular carcinoma. ER stress is sensed by a coordinated signaling pathway, initiated by signal transducers that span the ER membrane. UPR signaling consists 3 major sensors and their downstream pathways, protein kinase R-like endoplasmic reticulum kinase (PERK), inositol requiring enzyme 1 alpha (IRE1α, ubiquitous), inositol requiring enzyme 1 beta (IRE1β, tissue specific) and activating transcription factor 6 (ATF6) (Figure 1). Molecular chaperone BiP/GRP78 consistently and dynamically binds and arrests them in inactive status in the ER membrane. ER stress triggers the dissociation of these three major sensors from BiP. Released sensors undergo a conformational change necessary for activation and downstream signaling.

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As a central coordinator, the transducers sense stress and initiate a signaling pathway that makes the final life-and-death decisions for the cells undergoing variable stresses. ER stress signaling can restore homeostasis by reducing global protein translation, clearing the misfolded proteins, increase proper protein folding capability, facilitating cell adaption and survival. In contrast, ER stress can also initiate apoptosis by activating cell death genes during unmitigated stress. The competence of cell survival to ER stress is therefore proportional to the intensity and duration of the stress, the extent of the activation of the transducers, the execution of the downstream of effectors, the balance between pro-survival signaling and pro-apoptotic signaling (Chevet et al., 2015).

UPR Signal transducers

IRE1

IRE1 is a type I single pass transmembrane protein that undergoes oligomerization and trans-autophosphorylation under ER stress. IRE1 contains an RNase domain that selectively degrades mRNA, microRNA and ribosomal RNA in an IRE1 dependent decay (RIDD) way. Xbp1 mRNA, a transcriptional regulator, is the major substrate of IRE1. Activation of Xbp1 is the direct result of IRE1-dependent excision of a 26 nucleotide intron from the Xbp1 mRNA thereby generating a spliced Xbp1 mRNA that is more efficiently translated. Spliced Xbp1 modulates gene expression engaged in protein folding, secretion and endoplasmic reticulum-associated degradation (ERAD)(Hetz et al., 2011).

In budding yeast, a single IRE1 is expressed; while yeast IRE1 also harbors protein kinase activity, trans-autophosphorylation is its primary function and this contributes exclusively to its RNase function. As such, enforced dimerization which permits RNase activation is sufficient for IRE1 function (Ron and Hubbard, 2008). In contrast, the function of IRE1 in mammalian cells is more complex and perhaps as a reflection of this, higher eukaryotes harbor two IRE1 genes; IRE1α which is ubiquitously expressed, essential gene and IRE1β which is expressed in a tissue specific manner (Bertolotti et al., 2001; Wang et al., 1998). Unlike yeast, mammalian IRE1 has validated protein kinase substrates that include JNK (Urano et al., 2000) and Ask1 (Nishitoh et al., 2002) via Protein adaptor TRAF2. Phosphorylation of such substrates in turn contributes to cell adaptation to chronic versus acute stress (Lin et al., 2007).

ATF6

ATF6 activation requires export from the ER and translocate to the Golgi apparatus. Under ER stress, ATF6 is translocated to Golgi and processed by protein phosphatase S1P and S2P, where the cytosolic domain of ATF6 goes to nucleus and initiate a specific transcription program involved in protein folding and quality control (Haze et al., 1999; Sitia and Braakman, 2003). ATF6 may contribute to cancer cell dormancy and drug resistance, due to its cell adaptive function (Martino et al., 2013; Schewe and Aguirre-Ghiso, 2008).

PERK

While PERK like IRE1 is a single pass type I protein kinase, it contrasts with IRE1 in that it is a dedicated protein kinase. Analogous with IRE1, titration of BiP by misfolded proteins

permits PERK oligomerization, autophosphorylation and consequent phosphorylation of downstream substrates. PERK can phosphorylate a growing number of substrates including eIF2α (Harding et al., 1999), Nrf2 (Cullinan et al., 2003) and FOXO (Zhang et al., 2013), and the lipid diacylglycerol (DAG) (Bobrovnikova-Marjon et al., 2012; Pytel et al., 2015) (Figure 2). One of the key regulatory functions of PERK is its role as a regulator of protein translation; a reflection of direct phosphorylation of translation initiation factor 2α (eIF2α). eIF2α regulates the binding of the methionyl tRNA to the ribosome. Phosphorylation of eIF2α at Ser51 disrupts activation of the 43S translation initiation complex formation, thereby reducing the rate of general protein translation (Harding et al., 1999). The PERK-dependent reduction of protein translation is thought to limit nascent protein transport to ER lumen reducing potential chaperone load thereby permitting chaperones to clear misfolded aggregates. Intriguingly, translation of select proteins is increased; examples ATF4 and cIAP1/2 and, due to the presence of an upstream short open reading frame (uORF) (Hamanaka et al., 2009; Yaman et al., 2003). C/EBP homologous protein (CHOP), activated by ATF4 accumulation, plays multiple function as a transcriptional factor in ER stress signaling pathway, such as apoptosis and autophagy. For instance, CHOP activates proapoptotic Bax, leading to mitochondria mediated apoptosis (Szegezdi et al., 2006); CHOP and ATF4 cooperatively modulate expression of genes encoding regulators of autophagy including p62, Atg5, Atg7, and Atg10 (B'Chir et al., 2013; Szegezdi et al., 2006). In addition, ATF4 triggers increased expression of GADD34, a component of the PP1 phosphatase that dephosphorylates eIF2α (Ma and Hendershot, 2003), thereby mediating a negative feedback loop that limits PERK signaling.

PERK-dependent phosphorylation of nuclear factor erythroid 2-related factor 2 (Nrf2) on Threonine 80 promotes Nrf2 dissociation from Keap1, a scaffolding protein that contributes to Nrf2 degradation, thereby allowing Nrf2 translocation to the nucleus and activation downstream. Nrf2 as a master transcriptional factor of redox homeostasis limits ROS damage and renders cells chemoresistance (Cullinan et al., 2003) (Figure 2). The target genes of Nrf2 include antioxidant enzymes NQO1, GCLC et al., and drug transporters such as MRP2, MRP3 et al., conferring Nrf2 crucial function to maintain the redox homeostasis and resistance to chemotherapy (Ma, 2013).

A third PERK substrate FOXO1 is phosphorylated on S243 in *Drosophila* (Zhang et al., 2013) (Figure 2); this residue is conserved in human FOXO1 within cluster of phosphorylatable serines, Ser298, Ser301, Ser308 and Ser311 cluster, which regulates the nuclear translocation of FOXO1 (Zhang et al., 2013). Interestingly, PERK also regulates FOXO1 activity indirectly by participating in AKT activation, which is an upstream negative regulator of FOXO1 activity (Bobrovnikova-Marjon et al., 2012). The direct and indirect regulation of FOXO activity by PERK generates a negative feedback mediating insulin resistance and other functions.

Finally, PERK is also known to possess lipid kinase activity, phosphorylating the lipid diacylglycerol (DAG) (Figure 2) (Bobrovnikova-Marjon et al., 2012). DAG is the precursor to generate a central node lipid second massager phosphatidic acid (PA), which engages multiple mitogenic pathways and promotes tumorigenesis (Bruntz et al., 2014).

Oncogenic drivers, the UPR, and PERK

Tumors must proliferate and survive in a microenvironment that should be incompatible with rapid growth and expansion. The tumor environment is typically poorly vascularized resulting in limitations in key nutrients including oxygen, glucose, and growth factors (Harris, 2002). Such limitations trigger a number of stress responses, including the UPR (Figure 1). While engagement of these pathways can trigger cell death and prevent accumulation of damaged cells or simply aberrant growth, pathways such as the UPR are also adaptive and can be co-opted to enhance tumor cell survival and ultimately tumor progression. Such a process can be considered “non-oncogene addiction” and led many to consider whether such adaptive pathways are potential therapeutic targets. In this section, we focus on the contributions of PERK to tumor progression.

c-Myc is a potent oncogene that drives tumorigenesis in part through its capacity to increase ribozyme expression, biogenesis and ultimately protein synthesis (Grandori et al., 2005). As a result, c-Myc expression is associated with a dramatic increase in PERK activity both in mouse models and human lymphoma that harbor a c-Myc translocation (Hart et al., 2012). Myc expressing cells appear exquisitely dependent upon PERK-dependent autophagy and in the absence of PERK the reduction in autophagy favors apoptosis. PERK activity regulates ULK1 and ATG5 expression, both of which are essential for sustaining the high level of autophagy. This evidence couples the c-Myc increased protein synthesis and the activation of PERK-eIF2α-ATF4 UPR branch, suggesting UPR plays essential roles for c-Myc mediated cell survival and tumor progression via induction of cytoprotective autophagy.

BRAF/NRas mutations are the most common driver mutations in melanoma (Pollock et al., 2003). Oncogenic $BRAF^{V600E}$ triggers chronic ER stress, resulting in higher basal level of autophagy and resistance of apoptosis in melanoma. In melanoma, IRE1/ASK1/JNK accounts for autophagy induced by $BRAF^{V600E}$ (Corazzari et al., 2014). $BRAF^{V600E}$ also activates PERK as demonstrated by eIF2α phosphorylation and ATF4 induction in melanoma cell lines. Whether PERK contributes to the pro-survival autophagy in the BRAF mutant melanoma cells is not clear (Corazzari et al., 2014). One attractive hypothesis would be that PERK mediated autophagy and facilitates $BRAF^{V600E}$-dependent tumorigenesis in melanoma. Indeed, a second group addressed this question in the context of $BRAF^{V600E}$ inhibitor resistant melanoma cells (Ma et al., 2014).

$BRAF^{V600E}$ inhibition by vemurafenib represents a major advance in the melanoma treatment; unfortunately, almost all the patients got relapsed eventually and developed the $BRAF^{V600E}$ inhibition resistance (Flaherty et al., 2010). $BRAF^{V600E}$ inhibitor treatment triggers translocation of mutant $BRAF^{V600E}$ to the ER resulting in sequestration of BiP/Grp78 thereby activating PERK. PERK in turn was suggested to mediate through cytoprotective autophagy (Figure 1). Ultimately, PERK inhibitors appear to sensitize $BRAF^{V600E}$ mutant melanoma cells to $BRAF^{V600E}$ inhibitor treatment (Ma et al., 2014). One might speculate that combining vemurafenib with a PERK inhibitor or an autophagy inhibitor might produce robust clinical efficacy.

The HER2/Neu oncogene can also induce PERK in the context of mammary carcinoma. In mouse models of HER2/Neu-dependent mammary adenocarcinoma, deletion of PERK reduced growth of both the primary tumor and reduced metastasis by 50% (Bobrovnikova-Marjon et al., 2010). In this model, PERK loss had no impact on tumor incidence suggesting it is required for progression rather than transformation. The impact on progression was attributed to maintenance of redox homeostasis, via Nrf2, in this system rather than autophagy (Bobrovnikova-Marjon et al., 2010).

PERK impacts accumulation of select oncoproteins and tumor suppressors

Initial work on ER stress revealed a close association with cell cycle arrest. Subsequent work demonstrated PERK-dependent signals mediate arrest in G1 phase; a feature of reduced cyclin D1 translation (Brewer and Diehl, 2000; Hamanaka et al., 2005). PERK activation and eIF2α phosphorylation suppresses general protein translation, including cyclin D1 translation. Due to its short half-life, cyclin D1 expression is greatly attenuated during ER stress. Attenuated expression of cyclin D1 causes the impaired activity of cyclinD1-CDK4 complex, followed by $p21^{Cip1}$ redistribution to and inhibition of CDK2 thereby ensuring cell cycle arrest at G1 phase. Enforced expression of cyclin D1 mutants that are refractory to ubiquitin-dependent degradation, overcomes cell cycle arrest triggered by ER stress (Brewer et al., 1999) but in so doing sensitize cells to apoptosis.

Independent of cyclin D1, PERK mediated UPR triggers p53 accumulation and cell cycle arrest via ribosomal subunits: Hdm2 interaction (Zhang et al., 2006). PERK mediated eIF2α phosphorylation causes the reduced polysome formation and it is likely also increased free ribosomes. Therefore, these free ribosomal proteins, specifically rpL5/rpL11/rpL23 bind Hdm2 and sequester Hdm2 in the nucleus, which causes the impaired E3 ligase activity of Hdm2 and consequently p53 turnover slowdown. Accumulation of p53 also contributes to $p21^{Cip1}$ induction and cell cycle arrest in G1/S phase. Biogenesis of ribosome is energy consuming in physiological condition. It is reasonable to speculate that cells under harsh condition such as nutrition deprivation develop the strategy shutting off general protein synthesis, ribosome biogenesis and stopping cell cycle progressing to obtain an opportunity restoring the homeostasis. p53 accumulation beyond key threshold will trigger apoptotic gene expression, such as PUMA and NOXA, and inducing apoptosis (Li et al., 2006). ER stress has also been suggested to accelerate p53 degradation via GSK3β (Pluquet et al., 2005; Qu et al., 2004). Still other work suggests that ER stress leads to an initial downregulation of p53 followed by its recovery at later stages (Thomas et al., 2013). Emerging evidence suggests ER stress and PERK induces p47, a N-terminal truncated p53 translated from the 2^{nd} translational initiation site in p53 mRNA. PERK activation initiates a cap-independent p53 translation and consequently accumulating p53/47. p47 in turn triggers arrest in G2 phase (Bourougaa et al., 2010). Ultimately, p53 contributes to regulation of the G1/S transition cell cycle, while p47 regulates the G2 cell cycle transition via upregulation 14-3-3σ.

Does UPR activation and p53 regulation impact cell cycle checkpoints? Indeed, ER stress mediated protein translation attenuation has been implicated in Chk1 phosphorylation (Thomas et al., 2013). Since p53 is phosphorylated and activated by Chk1 (Polager and

Ginsberg, 2009), and p53 can impact recovery from protein translation inhibition by modulating GADD34, the phosphatase of eIF2α, p53 mediated G1/S arrest and Chk1 mediated G2 phase transition interactively affect each other. The G2/M and G1 arrest could occur sequentially during ER stress (Thomas et al., 2013). Taken together, PERK mediated cyclin D1 translation attenuation, p53 and its downstream cell cycle effectors (such as p21), p53 isoforms p53/p47 and Chk1 modulate cell cycle exit in response to ER stress and ultimately contribute to tumor cell fate.

PERK and tumor angiogenesis

Angiogenesis a process necessary for expanding the vascular network forming and provide the area of adequate oxygen and nutrients, is a crucial step for tumor development. The level of neoplastic angiogenesis reflects the degree of aggressiveness of tumors (Nishida et al., 2006). Anti-angiogenic inhibitors have been developed as potential therapeutic agents, targeting angiogenesis alone does not have long term beneficial for survival (Nishida et al., 2006). Work from a number of groups has linked PERK with tumor angiogenesis. PERK+/+ tumors exhibit microvessel formation from endothelia cells, while PERK−/− tumors have reduced vasculature (Blais et al., 2006). One key factor that may contribute is VCIP (VEGF and type I collagen inducible protein). VCIP plays pivotal roles in VEGF and bFGF induced capillary morphogenesis (Wary and Humtsoe, 2005). Cells under hypoxia express VCIP via translational regulation through 5’UTR internal initiation, which is exclusively dependent upon PERK, highlighting the importance of PERK pathway in tumor angiogenesis under hypoxia condition (Blais et al., 2006).

Remarkably, evidence emerged recently demonstrated that the pro-angiogenesis factor VEGF could induce UPR response via PERK and ATF6 to promote endothelia cell survival and angiogenesis (Karali et al., 2014). Interestingly, in this case, regulation reflects ER stress-independent UPR signaling through the PLCγ and mTORC1. VEGF rapidly activates all three UPR mediators without the displacement BiP from UPR transducers. PLCγ inhibitors, PLCγ deletion and mTORC1 inhibition effectively inhibited PERK, IRE1 and ATF6 activation. It has been proposed that PLCγ may bind to mTOR triggering its translocation to the ER membrane (Karali et al., 2014; Wang and Kaufman, 2014). The UPR signaling induced by VEGF is essential for the endothelia cells maintain the mTORC2 activity and ensues the AKT phosphorylation on Ser473. As a result, the VEGF phosphorylates AKT on Thr308 via PI3KPDK1 pathway, phosphorylates AKT on Ser473 via PLCγ/mTORC1/ATF6 PERK/mTORC2, achieving the maximal activation of AKT. Overall, VEGF induced ATF6 and PERK, but not IRE1 activation, promote endothelia cell survival and ensure its other functions. Of note, the CHOP activation, which represents the proapoptotic aspect of PERK signaling, is undetectable (Karali et al., 2014). Based on this, it is reasonable to speculate other growth factors in the tumor microenvironment could induce cancer cell angiogenesis via UPR. The VEGF triggered UPR signaling extends the knowledge of physiological function of UPR components as well as the stress alleviation function under stress condition for tumor cells.

PERK adapts cancer cells microenvironmental stresses (low glucose-hypoxia)

Nutrients such as glucose, the key 6 carbon sugar, is relatively low in the tumor microenvironment a consequence of poor and disordered vascular structure (Gullino et al., 1967; Hirayama et al., 2009). Cancer cells are highly addicted to glucose utilization, a consequence of the Warburg effect. This effect can be attributed to a shift from oxidative phosphorylation to glycolysis; this shift to glycolysis is now known to provide key substrates for anabolic metabolism while maintaining high energy for cell growth and proliferation (Vander Heiden et al., 2009). In this context, activated PERK can facilitate cancer cell survival under low glucose condition partially by AKT activation and hexokinase II mitochondria translocation (Hou et al., 2015).

In the solid tumor microenvironment tolerance to hypoxia is crucial for tumor cell survival. The oxygen concentration around solid tumor microenvironment and within the tumor proper is variable again a reflection of poor vasculature or dynamic blood flow. Hypoxia triggers reprogramming of metabolic gene expression which in turn reduces oxygen consumption and improves energy utilization. The hypoxic response is largely dependent on HIF-1 and HIF-2 (Ratcliffe, 2007). In addition to HIF mediated hypoxia adaption, the global protein synthesis reduction modulated by UPR is crucial to coordinate the hypoxia tolerance (Koumenis et al., 2007).

Hypoxia induces the PERK-eIF2α axis and loss of PERK compromises tumor cell survival under hypoxia condition *in vitro* (Koumenis et al., 2002). Additionally, tumor xenograft studies revealed that hypoxia triggers PERK signaling. Dominant negative PERK or eIF2α Ser51A impairs tumor growth in xenograft models and this correlates with increased apoptosis in hypoxic areas of the tumor (Bi et al., 2005). Additional evidence linked UPR responses to protection of tumor cells from hypoxia via induction of MAP1LC3B and ATG5 linking PERK-dependent regulation of autophagy with the hypoxic response (Kouroku et al., 2007; Rouschop et al., 2010). Increased expression of MAP1LC3B and ATG5 is dependent upon ATF4 and CHOP. Direct analysis revealed that both CHOP and ATF4 bind directly the respective promoters, leading to their induction in mRNA level. Collectively, these studies suggest that PERK signaling mediated hypoxia tolerance in tumor development. Targeting PERK pathway may sensitize hypoxia tumor cells to apoptosis.

PERK also stimulates tumor migration and metastasis under hypoxia conditions (Nagelkerke et al., 2013). Tumors under hypoxia condition tend to be more metastatic and malignant, correlated with poor prognosis (Chan and Giaccia, 2007). Whether PERK facilitates hypoxia activated metastasis becomes intriguing. Lysosomal associated membrane protein3 (LAMP3) has been identified greatly induced under hypoxia condition, mediating metastasis in breast cancer, cervix cancer and multiple cancer types (Mujcic et al., 2013; Nagelkerke et al., 2013; Nagelkerke et al., 2014). PERK mediated UPR mediates LAMP3 expression, where knockdown of PERK, ATF4 or overexpression GADD34 all compromised LAMP3 expression under hypoxia condition (Mujcic et al., 2009) suggesting a direct link between PERK and hypoxia stimulated metastatic spread.

Of note, there is increasing evidence suggests that autophagy is pro-survival for cancer cells within the tumor microenvironment (Amaravadi et al., 2011). PERK-dependent reprogramming of autophagic gene expression to promote cancer cell survival, adapts cells to harsh conditions such as hypoxia and oxidative tumor microenvironment, induces vascularization and migration, confers cancer cells aggressiveness and chemoresistance. Taken together, the autophagy induced by PERK is crucial for PERK mediated tumorigenesis.

PERK and redox homoeostasis

In addition to regulation of cytoprotective autophagy, PERK also regulates cell redox homeostasis. In the context of ROS accumulation within tumors, PERK action is clearly adaptive (Bobrovnikova-Marjon et al., 2010). PERK deficiency in the cancer cells results in increased ROS accumulation, a consequence of reduced Nrf2 activation. Nrf2, a direct PERK substrate, maintains the redox homeostasis by regulating expression of the enzymes (eg. NQO1 and GCLC) which produce antioxidant glutathione (Cullinan and Diehl, 2004; Cullinan et al., 2004; Cullinan et al., 2003). Nrf2 activation provides a cytoprotective effects on cells to counteract the ER stress perturbed cellular redox balance at the very beginning. Interestingly, CHOP depletes the glutathione and negatively correlated with Nrf2 expression, suggesting the Nrf2 may not mitigate the prolonged stress and ultimately cells commit to apoptosis (Cullinan and Diehl, 2004).

Regulation of Lipid biogenesis by PERK

Alterations in lipid metabolism is an under appreciated characteristic of tumorigenesis (Santos and Schulze, 2012). Work investigating the impact of PERK on mammary development demonstrated that PERK modulates expression of key lipogenic enzymes such as FAS, ACL and SCD1(Bobrovnikova-Marjon et al., 2008). Analysis of PERK function in the mammary gland revealed PERK-dependent control of lipid and free fatty acid production. With regard to mechanism, sterol regulatory element binding protein (SREBP1c), a major regulator of lipid metabolic genes, is induced and activated in a PERK-eIF2α dependent pathway. SREBP1c activation reflects PERK-dependent translational silencing of Insig1, a membrane anchor that prevents SREBP1 Golgi-translocation and processing (Bobrovnikova-Marjon et al., 2008). As discussed above, PERK possesses lipid kinase activity wherein it generates Phosphatidic acid (PA) through phosphorylation of diacylglycerol (DAG) as substrate (Bobrovnikova-Marjon et al., 2012). PERK lipid kinase activity is dependent on p85α subunit a regulatory subunit of phosphoinosotide kinase 3. PERK-dependent activation of AKT, mTOR and Erk1/2 in response to ER stress is dependent upon PA generation, highlighting the extensive roles of PERK in mitogenic signaling during tumorigenesis.

PERK-dependent regulation of metastasis

Tumor metastasis, a key aspect of the malignant phenotype, remains poorly understood at the molecular level and as such remains beyond current therapeutic intervention modalities. Most of the life threatening cancers experiences the invasive-metastatic cascade. Not

surprisingly given its capacity to regulate cell survival in response to microenvironmental stress, PERK, has been implicated in metastatic spread. PERK contributes to metastasis of HER2/Neu adenocarcinoma (Bobrovnikova-Marjon et al., 2010). While the underlying mechanism was not elucidated in this work, work from additional groups have proposed regulation of metastasis through the PERK-eIF2α-ATF4-LAMP3 axis in cervix cancer, breast cancer and head and neck squamous cell carcinoma (Mujcic et al., 2013; Nagelkerke et al., 2013; Nagelkerke et al., 2014).

Sustained PERK-eIF2α-ATF4 activation has been implicated in the Epithelia to mesenchymal (EMT) transition, a process that contributes to tumor progression and metastasis (Feng et al., 2014). Tumor cells acquiring the EMT, characterized as suppression of epithelia markers and upregulation of mesenchymal markers (such as snail and twist), become more invasive and drug resistant. Carcinoma cells with mesenchymal traits generate the properties of cancer stem cells, seeding more efficiently in both the primary and metastatic tumors (Mani et al., 2008). UPR signaling is activated during EMT process (Feng et al., 2014). Specifically, EMT triggers PERK-eIF2α signaling. The correlation between EMT and PERK activation also confirmed in primary breast cancer, colon cancer, gastric cancer, lung cancer as well as metastatic cancers spanning hundreds of clinical samples (Feng et al., 2014).

PERK contributes to chemoresistance

Multi-drug resistance remains an obstacle for chemotherapy. Amplification or mutation of ATP-binding cassette (ABC) transporters is implicated in increasing the efflux of drugs. Additional mechanisms for drug resistance include the upregulation of antioxidant enzymes, which could relieve the cancer cells from ROS produced from the chemotherapeutic chemicals. Nrf2 is a master regulator of antioxidant enzymes and glutathione biosynthetic enzymes (McMahon et al., 2001; Wang et al., 2008). Given that PERK regulates Nrf2, one might anticipate an impact on drug resistance. Indeed, PERK-Nrf2 axis associates with multi-drug resistance in de-differentiation cancer cells (Del Vecchio et al., 2014).

De-differentiation is associated poor prognosis and multi-drug resistance (Del Vecchio et al., 2014). De-differentiated cancer cells have constitutively pre-activated PERK-Nrf2 signaling, which is different from differentiated cells. In differentiated tumor cells, Nrf2 is actviated by oxidative stress through PERK mediated UPR pathway. However, in de-differentiated tumor cells, the PERK-Nrf2 is constitutive and activates a series antioxidant genes expression prior to the onset of oxidative stress, conferring cells with drug resistance. Evidence suggests that inhibition of PERK-Nrf2 could re-sensitize such cells to chemotherapy offering new avenues for therapeutic intervention.

PERK signaling through eIF2α may also contribute to drug and chemoresistance. PERK-eIF2α signaling is enhanced in chronic myeloid leukemia (CML) progression and associated with imatinib resistance. Blocking the PERK pathway impaires the proliferation and cologenic capacities of CML cells and sensitizes them to imatinib-induced apoptosis (Kusio-Kobialka et al., 2012).

Non-coding RNAs contribute to cell fate regulation by PERK

The investigation of small non-coding RNAs, also called microRNAs (miRNAs) has greatly advanced our knowledge of cancer biology. MicroRNAs play fundamental roles in many aspects of cancer development (Price and Chen, 2014) and the contributions of microRNAs to cell fate following ER stress have only recently gained attention. High throughput profiles on ER stress revealed that more than 80 microRNAs are significantly changed under ER stress, some of which contribute to the cell fate decision (Read et al., 2014). For example, miR-7a is upregulated in response to ER inducer thapsigargin and tunicamycin. The *in vitro* stimulated ischemia also induces both UPR response (confirmed by CHOP activation) and miR-7a expression (Read et al., 2014).

PERK regulates expression of two intronic micro-RNAs, miR-204 and miR-211(Chitnis et al., 2012). MiR-204 and miR-211 share the same seed sequence, although miR-204 is embedded within intronic sequence of the calcium channel trpm3, while miR-211 is embedded within intron 2 of trpm1. Unlike canonic microRNA that function by targeting 3'UTRs in the cytoplasm, miR-211/miR-204 transcriptionally regulate stress-dependent CHOP expression via the 5'UTR and promoter region. Here, miR-211/miR-204 nucleates a repressive complex referred to as RITS (RNA Induced Transcription Silencing) on the 5'UTR of rare CHOP transcripts. MiR-204 and miR-211 complexes contain an Argonaut protein and EZH2. Following miR-mediated binding to nascent transcript (Eulalio et al., 2008), this complex deposits repressive histone marks on promoter chromatin reducing RNA polymerase occupancy and reducing transcription. Intriguingly, the accumulation and turnover of miR-204 and miR-211 are rapid; offering cells a short window to antagonize CHOP mediated prematured apoptosis and restore the cellular homeostasis (Chitnis et al., 2012).

CHOP directly regulates microRNAs, including miR-708, which resides within the host gene Odz4 (Behrman et al., 2011). miR-708 serves as a potential therapeutic agent for metastatic breast cancer as it targets endoplasmic reticulum protein neuronatin to reduce calcium mediated cell migration (Ryu et al., 2013). However, whether PERK-CHOP regulates metastasis via miR-708 is elusive. miR-30c-2* regulates Xbp1 expression, induced by ER stress specifically through PERK pathway. This is another example for microRNA bridging the UPR pathways PERK and IRE1 crosstalk (Byrd et al., 2012). PERK also suppresses micro-RNA expression. miR-106b-25 cluster, as well as its host gene Mcm7, are negatively regulated by PERK-ATF4 and Nrf2 (Poliseno et al., 2010). miR-106b-25 cluster suppression contributes to apoptosis under severe ER stress, required for pro-apoptotic Bim activation (Gupta et al., 2012).

There is increasing attention in Long non-coding RNA (lncRNA) in cancer research, albeit with little knowledge of their function and working mechanisms. LncRNAs, RNA >200 bp nucleotides with no protein coding capacity, have the potential to play crucial roles in chromosome modification, transcription regulation and post-transcriptional modulation (Mercer et al., 2009). Recent work linked ER stress with expression of the lncRNA Malat1 (Bhattacharyya and Vrati, 2015). Flavivirus infection, which triggers UPR signaling in host cells, induces PERK-dependent Malat1 expression. Malat1, originally identified from

metastatic cancer cells, has been reported to promote tumor growth and metastasis in multiple cancers through regulating alternative splicing or binding to other transcription factors, such as EZH2 (Yoshimoto et al., 2015). Although the ER stress associated transcription factors for Malat1 and its target genes in the context of ER stress remain to be ascertained, given the neoplastic features of Malat1, we would anticipate that PERK induced lncRNA Malat1 might participate in PERK mediated tumorigenesis. More lncRNAs are waiting to be explored in the context of UPR and carcinogenesis.

Targeting PERK for cancer treatment

Genetic work revealing a prominent role for PERK in tumor progression, angiogenesis and metastasis, stimulated a search for small molecule PERK inhibitors with the hope of therapeutic efficacy. GSK2606414, a first generation of PERK inhibitor, targets PERK in its inactive DFG conformation at the ATP binding site. GSK2606414 inhibits PERK autophosphorylation *in vitro* and reduces growth of tumor xenograft (Axten et al., 2012). Second generation GSK2656157 showed modest improvement in pharmacological effects. Oral administration of GSK2656157 inhibited multiple tumor xenograft growth in a dose-dependent manner (Atkins et al., 2013; Axten et al., 2013).

The combination use of PERK inhibitor and proteasome inhibitor may also be a promising treatment for multiple cancers. Proteasome inhibitors such as carfilzomib and oprozomib exhibit significant clinical success in patients suffering from multiple myeloma and mantle cell lymphoma (McBride et al., 2015; O'Connor et al., 2005). Mechanistically, the proapoptotic impact of proteasome inhibitors is at least partially UPR-dependent (Dong et al., 2009; Nawrocki et al., 2005; Obeng et al., 2006). However, as PERK has pro-survival activities through ATF4 and Nrf2, PERK inhibition may foster improved clinical response (Obeng et al., 2006; Zang et al., 2012). It is attractive to speculate the combination use of PERK inhibitor could sensitize tumors to the proteasome inhibitors, reducing the dose of the drug and limiting the potential side effects.

While PERK inhibitors remain an exciting anti-tumor strategy, there are likely dose limiting toxicities associated with pancreatic damage (Figure 3). Genetic ablation of PERK triggers damage to both endocrine and exocrine pancreas resulting in a severe diabetic phenotype (Gao et al., 2012). Importantly, PERK function is required regardless of age. Support for this conclusion stems from eIF2α Ser51/Ala mutant knock-in mice which also exhibit severe glucose intolerance and diabetic phenotypes (Cavener et al., 2010).

While initial work suggested pancreatic damage observed in PERK deficient mice was cell autonomous, recent work has implicated non-cell autonomous IFN signaling in the deleterious effects of the PERK inhibition (Yu et al., 2015). IFNAR1, which encodes the interferon receptor1, is downregulated by PERK signaling. Global PERK deletion or pancreatic specific PERK deletion causes pancreas failure in mice in virtue of the high expression of IFNAR1 and hyperactivation of IFN signaling. Elevated IFNs are associated with pancreatitis and type I diabetes mellitus in human (Vassileva et al., 2003). Importantly, antagonizing the increased IFN receptor activity was suggested as a way to mitigate the PERK inhibitor deleterious effects. Indeed, the anti-IFNAR1 antibody relieved the

pancreatic toxicity, partially rescued the pancreas mass and stabilized glucose homeostasis in mice treated with PERK inhibitor GSK2606414 (Yu et al., 2015). A better understanding of the mechanisms of PERK inhibitor side effects is needed to develop the therapeutic strategy.

Concluding remarks

Great progress has been made in the past two decades to enhance our understanding of PERK contributions to cell fate and tumorigenesis. The contribution of PERK in cancer development is complicated, providing both the pro-survival and also pro-apoptotic signaling. It is not clear what dictates PERK pro-apoptotic signaling from pro-survival signaling. PERK function is dependent on tissue types and the microenvironment; for example, PERK excision did not affect the proliferation of mammary epithelia cells, whereas it potentiates tumor cell expansion (Bobrovnikova-Marjon et al., 2010). Moreover, PERK function differs greatly in distinct tumor stages. For example, PERK serves as a barrier for $HRas^{G12V}$ driven melanoma initiation (Denoyelle et al., 2006), but promotes cytoprotective autophagy in the melanoma progression and chemoresistance (Ma et al., 2014). The amount of active PERK may also determine inclusion of specific the downstream effectors and output. Furthermore, the crosstalk among PERK, IRE1 and ATF6 also comprehensively integrates the stress signaling and the output is dependent on the coordination among these three downstream pathways and their effectors.

Current evidence supports a model wherein PERK contributes to genomic instability, adaption to tumor microenvironment, aggressiveness and chemoresistance. For these reasons, PERK mediated cancer development integrates signaling networks and involved in multiple physiological functions. PERK inhibitors exhibit the potential in the anti-tumor therapies, but the minimization of side effects has to be further delineated. A deeper understand of how to combine PERK inhibitors with other drugs to compensate the pancreatic cytotoxicity is fundamental important for a viable cancer therapy.

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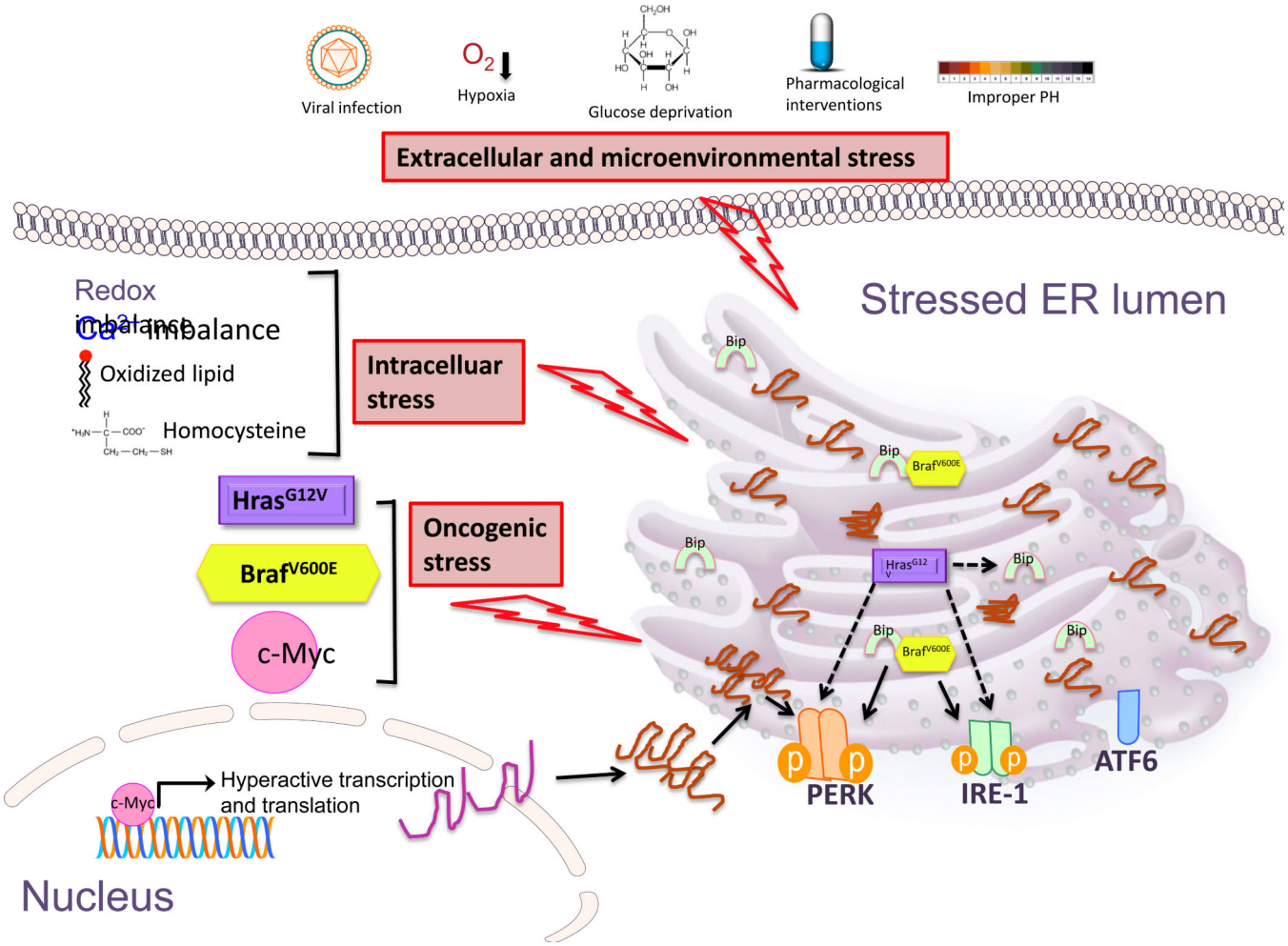

Figure 1. Activation of the UPR

Extracellular stress, intracellular stress as well as oncogene activation trigger ER stress and activate the transducers PERK, IRE-1 and ATF6. Extracullar stress includes viral infection, hypoxia, glucose deprivation, improper PH and pharmalogical interventions. Chemotherapeutic agents such as vemurafinib, paclitaxel and bortezomib induce ER stress in cancer cells. Intracellular stress such as redox imbalance, calcium imbalance, oxidized lipid and homocysteine result in unfolded protein response. Oncogenic stress such as cmyc amplification, BRAF mutation and HRas mutation all trigger ER stress. c-Myc greatly enhances the global transcription and translation, which cause large amount unfolded protein accumulated in the ER and activate PERK signaling. $BRAF^{V600E}$ binds protein chaperon GRP78/Bip and dissociates Bip from the 3 transducers, activating IRE1 and PERK pathways. $HRas^{G12V}$ causes activation of PERK and IRE1, also increases the Bip expression via direct or indirect way, the deep mechanisms are still unknown.

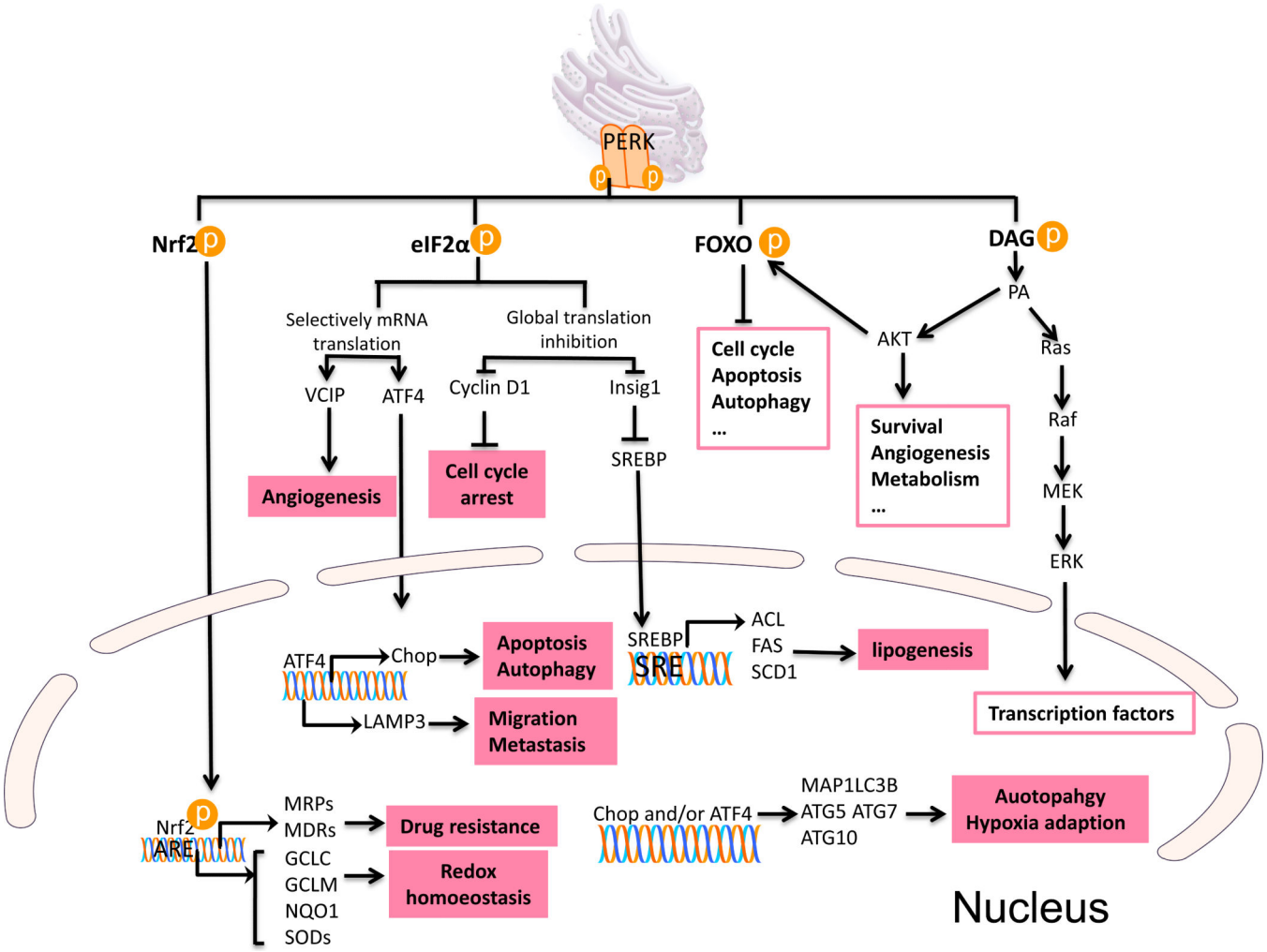

Figure 2. PERK signaling promotes cancer cell survival and aggressiveness

PERK phosphorylates its protein substrates Nrf2, eIF2α, FOXO and lipid substrate DAG. PERK phosphorylated Nrf2 dissociates from Keap1 and imported to nucleus, activating the transcription of drug resistance gene and redox enzyme genes. MRPs, multidrug resistance-associated proteins; MDRs, multidrug resistance proteins; GCLC, glutamate cysteine ligase catalytic subunit; GCLM, glutamate cysteine ligase modifier subunit; NQO1, NAD(P)H:quinone oxidoreductase 1; SODs, superoxide dismutases. PERK phosphorylated eIF2α exerts global protein inhibition, including cell cycle regulator cyclin D1 and lipid biosynthesis modulator insig1 (insulin inducible gene 1), which sequesters SREBP 1c (Sterol Regulatory Element Binding Protein) on the membrane to prevent its maturation. ACL, ATP citrate lyase; FAS, fatty acid synthase; SCD1, stearyl-CoA desaturase-1. eIF2α also selectively promotes some gene expression via alternative upstream open reading frame, such as ATF4 and VCIP. ATF4 activates CHOP transcription. ATF4, Chop alone or together regulate a broad range of genes, including those that regulate apoptosis, autophagy and hypoxia adaption. ATF4 regulated LAMP3 (Lysosomal-associated membrane protein 3) mediates migration and metastasis under ER stress. PERK activation of FOXO and phosphorylation DAG regulates the AKT function.

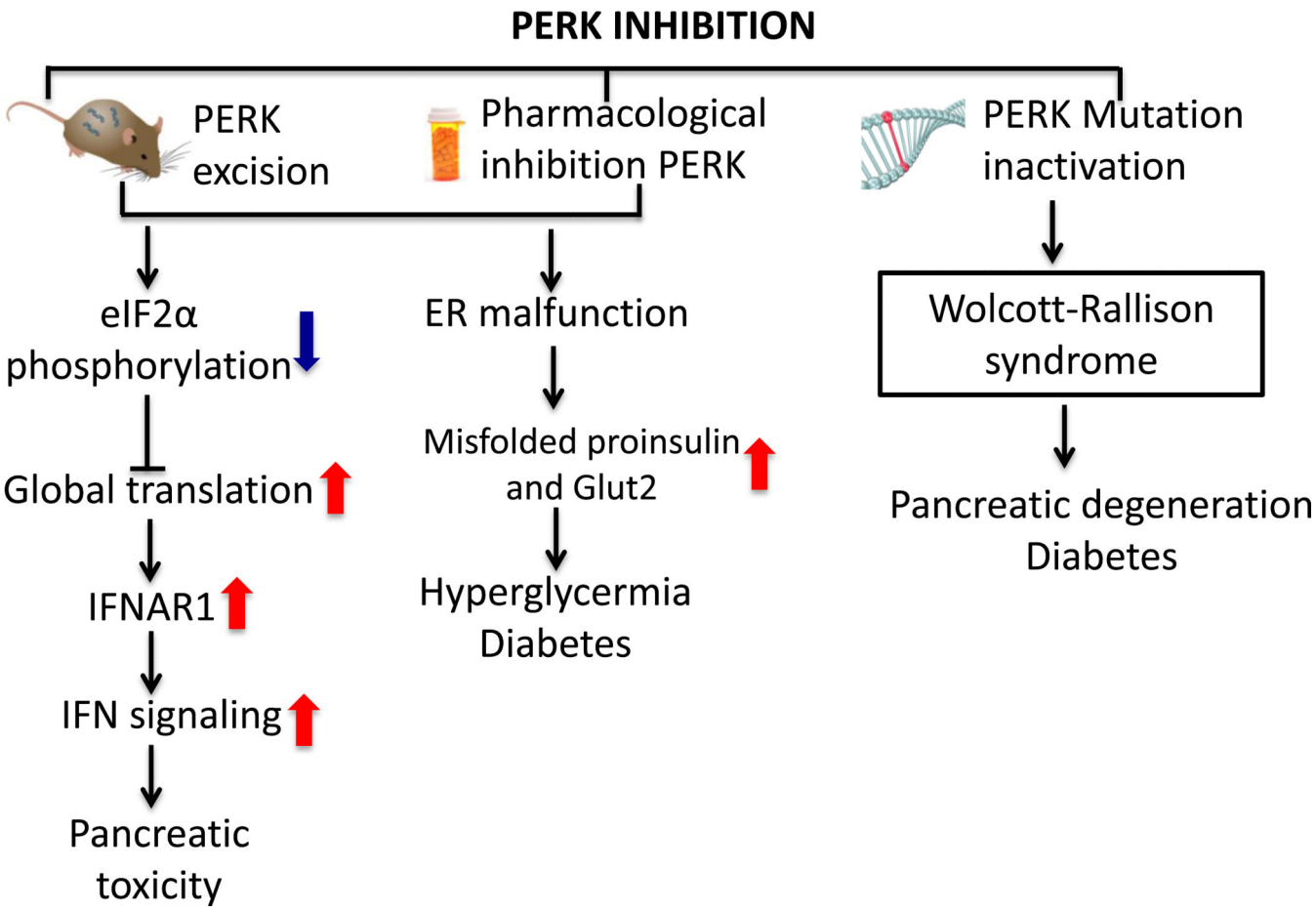

Figure 3. Pancreatic toxicity of PERK inhibition

PERK inhibition by either genetic deletion or pharmacological inhibition causes IFN signaling increase in pancreas and accumulation of misfolded insulin, proinsulin and glucose transporters Glut2, leading to hyperglycermia and diabetes. In human, PERK inactivation mutation results in Wlocott-Rallison syndrome, which is characterized with pancreatic degeneration and diabetes.